TOROGreen®
HRM qPCR Master Mix

Description

TOROGreen® HRM qPCR Master Mix(QET-100) is a Taq DNA polymerase based 2× master mix for use in qPCR applications and high-resolution melting (HRM) analysis, which contains the Hot Start Taq DNA Polymerase, PCR Buffer, dNTPs, Super EvaGreen® dye, ROX, Enhancer and Stabilizer. Except for using Super EvaGreen® instead of SYBR Green I, the master mix has the same composition as TOROGreen® qPCR Master Mix (QST-100). Therefore, it has all the features of QST-100. Due to the use of Super EvaGreen®, HRM analysis can be performed. Moreover, all primers will not form dimers in the NTC tests.

Feature

- **No primer dimer**

— all primers will not form dimers in the NTC tests.

- **HRM analysis**

— HRM analysis can be performed with the master mix.

- **Room-temperature stable**

— the performance is not easily decrease during storing and shipping.

- **Wide dynamic range**

— the mix demonstrates excellent reproducibility over a wide dynamic range and provides efficient amplification over 8 logs of sample.

No primer dimer

MIQE guidelines require NTCs should be included on each plate or batch of samples, and conditions for data rejection be established. In dye-based qPCR, non-specific amplification is mostly caused by primer dimers, and NTC control can check for the presence of non-specific amplification. As shown in figure 1, in the NTC experiment of a certain customer primer, qPCR Mix of brands N, T, A, and G showed non-specific amplification caused by dimers, while QET-100 did not form dimers.

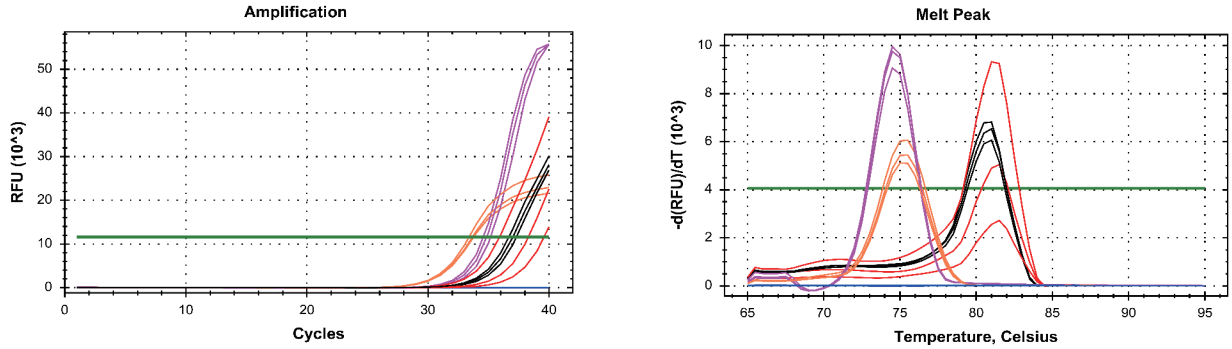

Figure 1. No primer dimer. NTC experiment was performed by customer using a certain primers. QPCR mix of brands N (red line), T(purple line), Y(orange line), and G(black line), showed non-specific amplification caused by dimers, while TOROGreen® HRM qPCR Master Mix (QET-100, blue line) did not form dimers.

HRM analysis

The genomic DNA of the Aope gene model mice was amplified using TOROGreen® HRM qPCR Master Mix(QET-100). The results of the melting curve were used to genotype the mice, as shown in figures 2. It shows that the fluorescence values are divided into three peaks, representing wild-type, heterozygous, and homozygous mice. By adding the saturated fluorescent dye Super EvaGreen, TOROGreen® HRM qPCR Master Mix can perform high-resolution melting curve analysis, replacing the use of partial probe fluorescence quantification methods such as SNP sites recognition and gene typing, greatly reducing experimental costs.

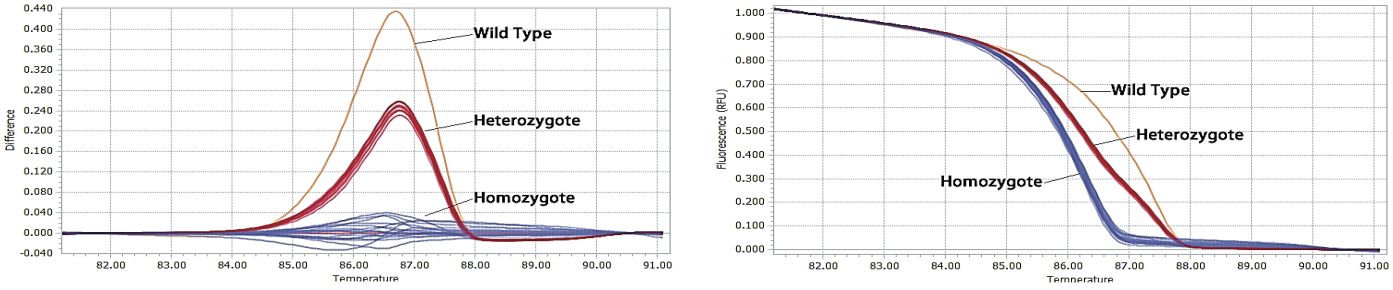

Figure 2. HRM analysis. The genomic DNA of the Aope gene model mice was amplified using TOROGreen® HRM qPCR Master Mix(QET-100). The results of the melting curve were used to genotype the mice.

Room-temperature stable

Extensive stability testing was performed on eight 10×dilutes of the template. TOROGreen® HRM qPCR Master Mix (QET-100) were sealed and left at 37℃ for 7days, and all results calculated and collated. From the amplification plot(Fig. 3), it shows that the QET-100 stored at 37℃ and at -20℃ have the same curve, and the Ct value is basically similar. QET-100 has extremely high stability within a wide range of template concentration.

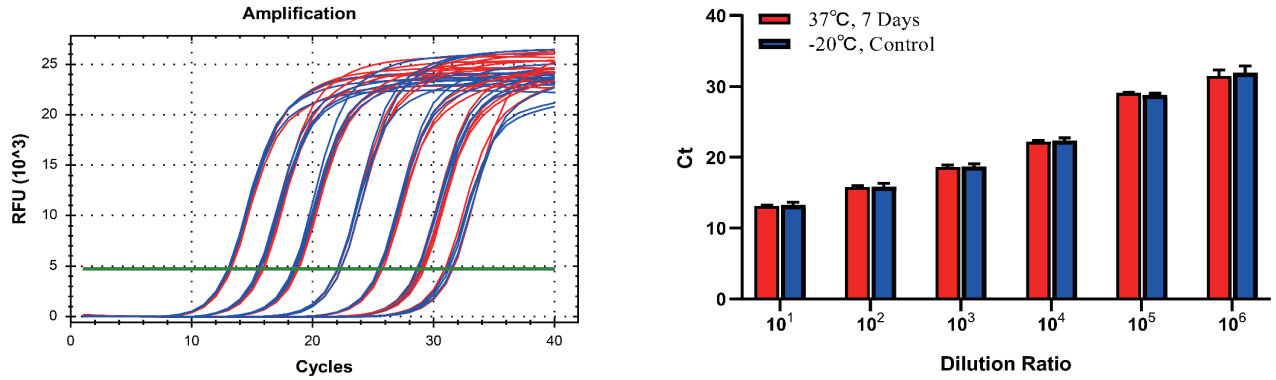

Figure 3. High stability. Real-time PCR was performed using pET28a plasmid with Bacillus badius phenylalanine dehydrogenase gene and primerss (F: AGGAAGCCGATGTGTTCGTT; R:TTCCGCTTGCTGGTACACTT) targeting pdh (phenylalanine dehydrogenase). TOROGreen® HRM qPCR Master Mix (QET-100) stored at 37℃ (red line) and at -20℃ (blue line) have the same curve, and the Ct value is basically similar.

Wide dynamic range

TOROGreen® HRM qPCR Master Mix (QET-100) is able to accommodate a wide range of input DNA/cDNA without compromising PCR efficiency. The Kanamycin resistance gene was amplified from a 10-fold dilution series of pET-28a plasmid to demonstrate the superior range and amplification efficiency of the QET-100. The amplification plot and standard curve (Fig. 4) show that the TOROGreen® HRM qPCR Master Mix displaying superior dynamic range and efficiency.

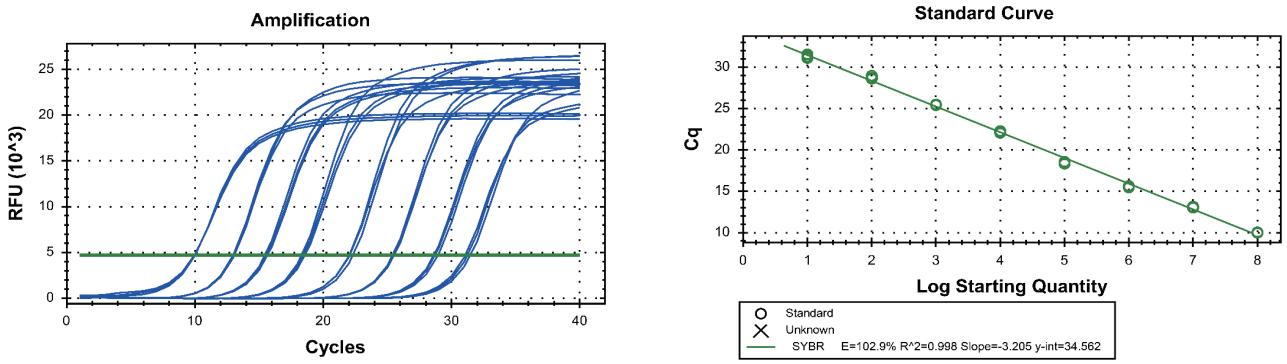

Figure 4. Wide dynamic range. Real-time quantitative PCR of 10-fold serial dilutions of pET-28a plasmid were performed using primers specific to the Kanamycin resistance gene with TOROGreen® qPCR Master Mix (QET-100). The amplification plot and standard curve show that QET-100 displaying superior dynamic range and efficiency.

Ordering information

Catalog Number	Product Name	Unit Size
QET-100	TOROGreen® HRM qPCR Master Mix	1 mL ×10 tubes

References
[1] Bustin SA, Benes V, Garson JA, etc,al. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. ClinChem.2009,55(4):611-22.

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